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Discovery of new GPCR ligands to illuminate new biology

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Abstract

Although a plurality of drugs target G-protein-coupled receptors (GPCRs), most have emerged from classical medicinal chemistry and pharmacology programs and resemble one another structurally and functionally. Though effective, these drugs are often promiscuous. With the realization that GPCRs signal via multiple pathways, and with the emergence of crystal structures for this family of proteins, there is an opportunity to target GPCRs with new chemotypes and confer new signaling modalities. We consider structure-based and physical screening methods that have led to the discovery of new reagents, focusing particularly on the former. We illustrate their use against previously untargeted or orphan GPCRs, against allosteric sites, and against classical orthosteric sites that selectively activate one downstream pathway over others. The ligands that emerge are often chemically novel, which can lead to new biological effects.

GPCRs represent the largest class of signaling receptors in the genome, as well as the protein family most frequently targeted by therapeutic drugs (Fig. 1). GPCRs respond to various ligands, from protons to biogenic amines to lipids to chemokine proteins, and are involved in biological phenomena varying from cell division to bronchial relaxation to heart rate and blood pressure control, in addition to learning, memory, and cognition. Their attractiveness for drug discovery reflects the importance of the signals they transduce and the extracellular accessibility of their binding sites. Structural determination of almost 40 GPCRs in the last decade has revealed them to be well suited for small-molecule recognition —a post hoc explanation for their preponderance among drug targets.

Most GPCR drugs were discovered by combining classical medicinal chemistry with organ and cell-based pharmacology, decades before their targets were classified into a single family or even defined as true molecular entities[1] (Fig. 1). It has been estimated that 70% of GPCR drugs are analogs derived from the endogenous ligands of the receptors[2]; although this is not strictly true, the small chemical repertoire of early drug discovery, and the inability to counterscreen for specificity, ensured that many of the GPCR drugs resembled

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one another and were promiscuous. Whereas the resulting polypharmacology has sometimes contributed to efficacy[3,4], the lack of specificity of these older drugs has limited their usefulness as tools and has contributed to their side effects.

In the last decade, three discoveries have motivated the search for new GPCR chemotypes. First, it has become clear that GPCRs couple not only to their eponymous G proteins, but to other effectors as well, activating orthogonal pathways[5] (Fig. 2). This has inspired campaigns to find 'biased' agonists that preferentially activate one pathway over another. Second, the determination of pharmacologically relevant GPCR crystal structures[6] has revealed the binding sites of allosteric modulators and suggested new potential allosteric sites. Ligands that bind to these sites can either negatively or positively modulate endogenous transmitters with or without an intrinsic signaling effect of their own (Fig. 3). Third, the GPCR structures have enabled structure-based discovery and optimization of new ligands. Together, these developments have supported a renaissance in GPCR pharmacology and drug discovery.

Here we consider new approaches to finding tool molecules and therapeutic leads for GPCRs. These methods include physical assays that can interrogate most of the ~350 pharmacologically relevant GPCRs, including orphans, as well as structure-based docking screens that interrogate large compound libraries. We will focus more on the structure-guided approaches, as these are potentially scalable for use by a wide community and have received less attention among pharmacologists. A key contention of this Perspective is that the novel chemotypes discovered by these new technologies will often confer new biology, even against heavily interrogated targets. Although there is no single physical reason why this should be true, novel chemotypes may interact to stabilize one of the manifold of conformations available to GPCRs[7,8] in unique ways. This in turn can activate one of the multiple pathways downstream of the receptor with a specificity not previously explored by endogenous or synthetic ligands.

Structure-based docking screens

Molecular docking virtually screens large libraries of compounds for their fit into a receptor pocket. About 10^7 complexes are sampled, including thousands of orientations and conformations for each library compound, occasionally along with several snapshots of low-energy receptor conformations. Screens of several million molecules are common, and between 10^{13} to 10^{14} complexes may be sampled and scored. Docking scoring functions evaluate polar and nonpolar complementarity, steric fit, and solvation, among other terms. On an academic cluster, such a screen might be completed in several days of elapsed time (hundreds of core days).

This speed comes at the expense of accuracy, and docking scoring functions make grave approximations. The technique is unable to calculate binding affinities accurately, reliably rank order among high-scoring molecules[9], or typically differentiate agonists from antagonists. What a docking screen can hope to do is distinguish plausible ligands from the vast number of library molecules that will not bind (decoys). When drawn from commercially available molecules or from an in-house library, failure is cheap, and the

enterprise is worthwhile, as long as new and interesting molecules are found, notwithstanding the false-negatives.

A driver of this method has been the secular expansion of the docking libraries. These libraries reflect the integrated interests of the community over time, and are composed of molecules resembling metabolites, natural products, and drugs. Thus, docked molecules are not only readily acquired, but also typically far more bio-like than would be expected from a truly diverse, random library[10]. Over the last 25 years, the size of docking libraries of commercially available compounds have doubled every 2.5 years, and now approach 10 million accessible 'lead-like' molecules with favorable physical properties.

Both docking programs and their libraries are available to the community, many for free (for example, http://blaster.docking.org and http://zinc15.docking.org; see ref. 9). Admittedly, there remains an art to docking, including visual interrogation of top-ranking molecules from the library and hit-picking parties[9] that expose them to teams of medicinal chemists, structural biologists, and pharmacologists. Still, investigators can launch large library campaigns using community-available programs, libraries, and public access computational clusters from their desktops.

GPCRs as templates for docking screens

In the early 2000s, hit rates against soluble proteins in prospective, unbiased docking screens ran between 5%[11] and 35%[12], with hit rate defined as the number of compounds active on testing divided by the number physically tested. Occasionally, docking screens of receptor-focused libraries could enjoy even higher hit rates[13], and even then the technique had successes against GPCR homology models[14]. Still, certainly in our hands and especially for general library screens, the lower rates of 5–10% were more common, and against some targets the method failed entirely (and such outright failure can still occur today). Docking campaigns were most successful against tightly defined sites such as nuclear hormone receptors[15]. Encouragingly, the docking predicted structures that typically corresponded to the experimental result when co-complexes were determined by crystallography[11,16–18]. Still, with high false positive rates, and affinities often in the midmicromolar range, the reliability of docking for tool molecule discovery remains questionable.

Docking outcomes often have been better against GPCRs, with hit rates regularly above 20% and as high as 73%, and affinities occasionally in the sub- and often in the midnanomolar range (Table 1). Docking is typically more successful against neurotransmitter GPCRs, with their tighter orthosteric sites, than against peptide- and protein-binding GPCRs, but even against the latter, hit rates and affinities have been substantial. Compellingly, these screens have found new chemotypes even against well-established receptors. There is no single explanation for this improvement, but it may reflect (1) the well-formed binding sites of the receptors compared to those of soluble receptors; (2) bias in the libraries toward GPCR chemo-types[19]; and (3) the incremental improvement in docking methods over the last 15 years[9], with most of the GPCR docking screens occurring in the last six years. The combination of high hit-rates, high hit affinities, and hit novelty supports the pragmatism of

structure-based screens for new GPCR chemotypes. Remarkably, these new chemo-types often confer new patterns of GPCR signaling and biology.

Allostery and bias

By convention, the binding sites for endogenous ligands at GPCRs have been referred to as orthosteric, whereas binding sites that modulate orthosteric ligand activity have been called allosteric[20,21] (Fig. 3). The International Union of Basic and Clinical Pharmacology/British Pharmacological Society (IUPHAR) defines an allosteric site as a "binding site on a receptor macromolecule that is nonoverlapping and spatially distinct from, but conformationally linked to, the orthosteric binding site." A functional definition of allostery, meanwhile, emphasizes the saturation of the effect, with no further modulation seen past a certain concentration of the allosteric ligand. It also emphasizes the 'probe dependence' of allostery, with the same allosteric ligand having different effects on diverse orthosteric ligands. A fascinating aspect of allosteric modulators is that their actions may be only manifest in the presence of an endogenous signaling molecule or an orthosteric drug, whose signaling effects they amplify or dampen without a tonic effect of their own. The ability of allosteric potentiators to faithfully amplify the spatial and temporal aspects of native signaling makes them unique among drugs (further reviewed in ref. 7).

GPCRs recognize allosteric ligands at several distinct sites. The earliest allosteric modulator characterized was the negative allosteric modulator (NAM) sodium acting on the opioid receptor[22]. Since then, sodium has been recognized as a NAM for many family members, acting via a conserved pocket on the cytoplasmic side of the orthosteric site[23,24] (Fig. 3). Following agonist binding, G-protein engagement, and receptor activation, the sodium pocket collapses, relieving the negative allosteric brake on signaling. This sodium pocket might be targetable by small molecules extending from the orthosteric site.

Allosteric sites for drug-like molecules have also been characterized in GPCRs. In metabotropic glutamate receptors, both positive allosteric modulators (PAMs) and NAMs have been designed[25]. In smoothened, functionally allosteric modulators have been observed crystallographically in what would be part of the transmembrane orthosteric site in other GPCR families[26] (Fig. 3). In muscarinic receptors, a large open vestibule has been structurally characterized on the extracellular side of the orthosteric site[27,28], which is consistent with the binding of the PAMs and NAMs that have emerged from functional studies[29]. In the M2 receptor, this vestibule closes upon formation of a tertiary complex with a PAM and an orthosteric agonist (Fig. 3). A spectacular result of recent crystallography, and one case of a large DNA-encoded library screening[30], is that two other allosteric sites on GPCRs are targetable by small molecules: the intracellular region where G protein binds[31,32] and an intramembrane region on the outer surface of the helical bundle[33,34]. These sites appear conserved among family A GPCRs, suggesting that they may be broadly targeted. Admittedly, they are shallower than is typical of GPCR orthosteric sites and may be more challenging for ligand discovery.

Functional selectivity, or signaling bias, also reflects modulation of downstream signaling by ligands, here by favoring one of several possible pathways. As originally proposed[35] and

subsequently expanded upon[36,37], functional selectivity from a chemical biology perspective is “the ligand-dependent selectivity for certain signal transduction pathways in one and the same receptor.” From a coupling standpoint, functional selectivity could arise via differential activation of G-protein signaling (for example, G_i vs. G_s, as for the β2-adrenergic receptor[38]) or, more commonly, differential activation of β-arrestin (β-arr) compared to G-protein signaling[5] (Fig. 2a). From a structural standpoint, biased signaling reflects the multiple activated states through which a GPCR can transit that can be stabilized by different ligands. There are many possible effectors through which GPCRs modulate signaling, including several G proteins, β-arrs, and kinases, and each may be involved in different pathways. Thus, it is conceivable that for some GPCRs, each ligand could have a unique signature when multiple effectors are measured[39], and one could imagine ligands with downstream signaling signatures tailored for different outcomes. This may be exemplified at the 5-HT2B serotonin receptor, against which each ligand may be clustered by a signaling signature (Fig. 2b).

Several new tools allow high-resolution interrogation of allosteric modulation and functional selectivity, enabling new activity screens. A near GPCRome-wide β-arr screening platform, PRESTO-Tango[40], now available via ADDGENE (https://www.addgene.org/kits/roth-gpcr-presto-tango/), has been used by several labs to determine the extent of β-arr activation and bias for GPCRs[23,41–43]. In our own lab, the platform has been used to screen for ligands that illuminate the function of orphan and understudied GPCRs (oGPCRs)[40], such as MRGPRX2 (ref. 44).

An approach to discovering allosteric modulators in the absence of an orthosteric agonist, which is useful for illuminating oGPCRs, involves the overexpression of G proteins in yeast[45] and the tuning of their constitutive activity (Fig. 3d). Here we exploited the extended ternary complex model, which predicts that overexpression of GPCRs will potentiate basal GPCR activity. Because yeast G proteins interact poorly with mammalian GPCRs, systematic bias or crosstalk between endogenous and exogenous G proteins and GPCRs is minimized. The enhanced basal activity allows not only agonists, but also inverse agonists and allosteric modulators to be identified (Fig. 3d). We have used this system to discover initial hits for multiple oGPCRs, optimizing them into allosteric chemical probes for GPR68 and GPR65.

Several other approaches for interrogating GPCRs, including new G-protein and β-arr biased chemogenetic tools, have been recently introduced[46,47]. New conformationally sensitive β-arr sensors have been reported that may enable the unbiased identification of GPCR modulators, including small molecules and interacting proteins, that bias GPCRs toward different states of β-arr activation[48].

Case studies

From a receptor-centric perspective, new GPCR ligands can confer new biology in three ways. First, they can bind to an entirely new target. Second, they can bind to a new site on an established target, allosterically modulating endogenous transmitter or drug signaling. Finally, and most surprisingly, they can bind to an established site on an established target

and confer new biology by biased signaling, specifically activating one of multiple GPCR signaling pathways via preferential stabilization of one of many activated states (Fig. 2).

New chemistry for new targets

New biology is expected the first time a GPCR is modulated by a drug or reagent. Recent examples include the anti-HIV drug maraviroc, which targets CCR5; the anti-neoplastic vismodegib, which targets Smoothened; and molecules that have deorphanized GPCRs.

Maraviroc and CCR5

CCR5 is a co-receptor for HIV cell entry. As CCR5 is a membrane protein, a point of host–pathogen interaction, a protein–protein interface (PPI) target, and a receptor for which there were no previous drugs, it presented multiple technical challenges. A high-throughput screen (HTS) at Pfizer returned hits with ligand efficiencies that were substantially higher than the 0.24 kcal/HAC (heavy atom count) mooted as a bar for most PPI targets[49], consistent with the general advantages of GPCRs for small-molecule recognition. Advancement to maraviroc involved multiple rounds of chemical optimization to overcome hERG (human ether-à-go-go-related gene), a cardiac ion channel and metabolic liabilities. Though the crystal structure of the maraviroc–CCR5 complex shows that the drug partly overlaps with the orthosteric chemokine site[50], its probe dependence and its saturable noncompetitive binding functionally define it as a CCR5 NAM. Because the series leading to maraviroc emerged from target-based HTS, it bears little similarity to biogenic molecules, unlike many drugs from the premolecular era.

Vismodegib and Smoothened

Smoothened is a class F (Frizzled) GPCR that modulates embryonic development and tissue homeo-stasis as part of the hedgehog signaling pathway[51]. Protein mutations and epigenetic changes that hyperactivate this pathway are common in human tumors[52]. Vismodegib (GDC-0449), described by Genentech in 2009 (ref. 53), antagonizes Smoothened[54], inhibiting hedgehog signaling. Vismodegib was the first drug approved for this pathway and is used to treat late-stage basal cell carcinomas. Resistance to this drug arises from point mutations in Smoothened itself (for example, D473H), which abolish binding while maintaining protein activity[54]. As revealed by crystallography, substitutions at D473$^{6.55}$ have differential effects on antagonists[55], suggesting opportunities for structure-based discovery of new molecules that avoid this and other resistance mutations[56] that rapidly appear upon vismodegib treatment[57].

GPCR deorphanization

Because the new screening platforms (above) do not depend on knowing an agonist or displaceable ligand in advance, they have enabled the interrogation of understudied and oGPCRs[58]. Using yeast growth and PRESTO-Tango platforms, molecules may be screened against oGPCRs without knowing an agonist or function in advance. The libraries screened are often composed of privileged compounds—drugs and reagents—and hits reveal not only starting points for probes, but also previously hidden drug off-targets. For instance, a recent large-scale interrogation of oGPCRs by the PRESTO-Tango resource revealed that

nateglinide, a putatively selective potassium channel modulator, is a potent agonist of the oGPCR MRGPRX4. Similarly, saquinivar—a putatively selective HIV protease inhibitor—emerged as a potent agonist of the oGPCR BRB3 (ref. 40).

However, the drugs and reagents that make up the privileged library cannot themselves be used to probe oGPCRs biology, as they are almost always far more potent at their therapeutic targets. To leverage the initial hits for probe development, a combined experimental- and structure-based approach has been adopted. This can be illustrated by a deorphanization campaign against GPR68 (also known as OGR1)[45]. In a yeast-based screen, the GABAergic drug lorazepam was found to be a GPR68 PAM for protons, fortuitously present at higher concentrations in the low pH of the yeast screen. Several thousand structural models of GPR68 were calculated and prioritized by their ability to dock and highly rank lorazepam in comparison to the hundreds of nonbinding decoy drugs from the experimental screen. A cycle of docking, model refinement, and ultimately mutagenesis led to a consensus model for the lorazepam– GPR68 complex; this complex fit lorazepam preferentially over most of the decoys. Subsequently, a docking screen of 3.5 million commercially available molecules from ZINC[59] led to a compound dubbed ogerin (for OGR1 ligand), a potent and selective GPR68 PAM that has no activity for GABA or for other oGPCRs related to GPR68. Studies in wild-type (WT) and GPR68 knockout (KO) mice revealed that ogerin has on-target activity that suppresses contextual fear conditioning. This implies that GPR68 functions in brain pathways involving learning and memory[45]. Ogerin and an inactive analog are available as a probe pair for exploring GPR68 biology (http://www.sigmaaldrich.com/catalog/product/sigma/sml1482?lang=en&region=US;http://www.sigmaaldrich.com/catalog/product/sigma/sml1483?lang=en&region=US).

A similar screen has suggested that the oGPCR MRGPRX2 is an atypical opioid receptor, responding to drugs like morphine, codeine, and dextromethorphan, as well as to the endogenous opioid peptide dynorphin[44]. MRGPRX2 is expressed in mast cells and sensory neurons, consistent with its role as an opioid itch receptor. To find specific chemical probes for MRGPRX2, a cycle of docking and testing was again deployed, leading to ZINC-72453573 (ZINC-3573; Fig. 4), an agonist that has no detectable activity against the four opioid receptors, against 316 other GPCRs, or against a panel of 97 representative kinases. ZINC-3573 potently stimulates mast cell degranu-lation and may be used to further probe the function of MRGPRX2. ZINC-3573 and an inactive stereoisomer are available as a probe pair for exploring MRGPRX2 biology (http://www.sigmaaldrich.com/catalog/search?interface=All&term=(R)-ZINC-3573&N=0&focus=product&lang=en&region=US).

Variations on this screening strategy are being investigated. In an empirical screen of 5,472 Riken library molecules, inverse agonists for the SREB family of oGPCRs, including GPR173, were discovered, with affinities in the low-to-midmicromolar range[60]. These molecules were used to template the modeling of the GPR173 structure, akin to the workflow used for GPR68 and MRGPRX2, although here it wasn't used to find new ligands. A docking screen of 10,526 in-house compounds against a homology model of GPR171 (ref. 61) identified a dicarboxyphenyl vinyl amide, MS0015203 (ZINC4956098; MW 249), as an orthosteric agonist[61]. MS0015203 had activity *in vivo* in modulating feeding, which is

unusual for a primary fragment hit. This suggests a role for GPR171 in regulating appetite, feeding, and perhaps metabolism.

Together, these campaigns reflect a target-based screening approach to deorphanization. The goal is often to develop probe compounds that can illuminate the biological function of the orphan receptors, enabling further research. Many of the tools used are openly accessible to the community: the PRESTO-Tango platform is available from ADDGENE[45], and several of the docking tools and databases are free online[9]. An NIH project to deorphanize the drug-gable genome has created resources to associate orphan receptors with biology and disease (http://pharos.nih.gov/idg/about).

Nonscreening- and even nontarget-based approaches to GPCR deorphanization are also being explored. A structural strategy, CoINPocket, compares ligand contact residues of the orphan receptors to those of GPCRs of known activity[62]. When an orphan receptor has an orthosteric site and modeled interacting residues similar to those of the ligand-annotated receptors, it may inherit the known ligands and interactions of those receptors. This was the case for the oGPCR GPR37L1. Though the endothelin receptor (ET) is the closest characterized receptor to GPR37L1 by sequence, neither endothelin peptide nor synthetic ET ligands modulate the oGPCR. However, the modeled structure of the GPR37L1 orthosteric site resembled those of the bombesin, orexin, and neuropeptide S (NPS) receptors, correctly suggesting that the receptor would recognize orexin and NPS ligands such as ACT-335827, JNJ-10397049, and SHA-68 (ref. 62). A related approach provides a structural context that is based on homology modeling of most of the non-olfactory GPCRome (http://gpcrdb.org/) for pharmacophore-based discovery[63]. These homology models draw on the extensive ligand-binding and mutagenesis information often available for GPCRs to provide experimental restraints for the models[64]. The approach has found new dipeptide ligands for the oGPCR GPR139 (ref. 65), suggesting an endogenous ligand chemotype.

A less directed, but functionally powerful, strategy is target identification in phenotypic screens. These screens begin with compounds that have a cellular, or occasionally whole organism, phenotype, and seek the targets responsible for that activity. For instance, investigators found cyclohexylmethyl aminopyrimidines in a cell-based phenotypic screen for hedgehog pathway modulators. Gene expression analysis suggested that GPR39 might be the target, consistent with specific gene knockdown and ultimately on-target dose–response studies with optimized compounds. A role for this oGPCR in modulation of the hedgehog pathway protein Gli was suggested[66].

Allosteric ligands with new biology

Whereas the most obvious way that new chemotypes can confer novel biology is by modulating new targets, allosteric modulators can do the same by acting on new sites of established targets. Although most drugs act tonically, allosteric modulators may be used to act only in the presence of an endogenous ligand, increasing (PAMs) or decreasing (NAMs) native signaling (Fig. 3). One advantage of targeting allosteric sites is that they typically

differ more among receptor subtypes than do orthosteric sites, enabling selective ligands that are otherwise challenging to find.

Among the most established allosteric targets are the muscarinic receptors[67]. Despite their long-recognized role in central nervous system (CNS) disorders, muscarinic therapies for dementing diseases like Alzheimer's disease have been restricted by dose-limiting side effects, which are often mediated through other muscarinic receptor subtypes. The lower sequence identity of muscarinic allosteric sites has made it possible to find selective modulators. Leveraging the PAM LY2033298, Conn and colleagues discovered a submicromolar PAM of acetylcholine, VU0152100, that was specific for the M4 receptor subtype[68]. Rounds of synthetic optimization led to PAMs that are more CNS penetrant and stable *in vivo*[69] and that have interesting new biology, including modulation of sleep– wake disturbances associated with schizophrenia. Meanwhile, a compound from Merck, MK-6884, is in phase I trials as a potential positron emission tomography (PET) ligand to assess occupancy of M4 PAMs *in vivo* (NCT02621606).

The M1 muscarinic receptor has been implicated in synaptic plasticity[70], and M1 PAMs can enhance memory and cognition in animal models of Alzheimer's disease. Here too, orthosteric ligands lead to dose-limiting side effects via other muscarinic subtypes. The M1 PAM MK-7622 is currently in phase II clinical trials for add-on treatment with the anticholinesterase and D4 antagonist[71] donepezil (https://clinicaltrials.gov/ct2/show/NCT01852110). Here again, beginning with HTS hits[72], M1 PAMs were optimized for activity in animal models of schizophrenia. Intriguingly, and unlike the classic dopaminergic antipsychotics, the M1 PAMs improve cognition and reduce negative symptoms of psychosis in animal models[70]. The new muscarinic PAMs may act in concert with allosteric modulators of the metabotropic glutamate receptors, which are also increasingly targeted[25].

New biology via well-studied orthosteric sites

As it is obvious that ligands for novel GPCRs will confer new biology, and sensible that allosteric modulators will as well, perhaps the least intuitive observation has been the novel biology conferred by new chemotypes acting on well-explored orthosteric sites. This is surprising on two counts: one might not expect unrelated ligands to bind to the same site, and, if they did, one might think they would activate the same signaling pathway. There are, however, both empirical and theoretical reasons to expect unrelated ligands to bind to the same site, and signaling reasons to hope that they might confer new biology. Empirically, multiple unrelated classes of ligands have been shown to bind to the same site for multiple receptors and enzymes[11,73–77]. Conceptually, the apparent promiscuity of these sites is supported by the plasticity of molecular recognition, whereby the same functional group can be recognized by multiple different environments and the same binding site can recognize multiple different chemotypes[78]. For GPCRs, with their manifold of active and inactive states, finding new chemotypes against the same site is that much more plausible. The emergence of biased signaling offers a framework for understanding how a new chemotype might bind at a well-liganded orthosteric site, stabilize one of several activated states, and activate a subset of pathways.

Biased agonists for the μ-opioid receptor

An early and powerful example of biased signaling came from the work of Bohn and Carron on the μ-opioid receptor, which is the target of morphine and other opioid analgesics[79]. Morphine analgesia was unaffected by knockout of the β-arrestin2 protein in the mouse, but several of its side effects were attenuated, including respiratory depression and constipation. Inspired by this observation, HTS campaigns sought biased agonists that preferentially activated the G_i over the arrestin pathway. This led to the discovery of oliceridine (TRV130), a potent partial agonist of G_i signaling that only slightly activates arrestinergic signaling. As predicted by the knockout studies, oliceridine confers analgesia with little respiratory depression, nausea, or constipation at analgesic doses. Oliceridine is a novel chemotype for the μ-opioid receptor, with its closest known ligand having an ECFP4-based Tanimoto coefficient (*T*c) of only 0.29, consistent with the molecule representing a new scaffold. Though the new biology conferred by oliceridine could not have been predicted from its structure, the availability of reliable assays for biased signaling made the recognition and optimization of its activity possible. Oliceridine is now in phase III clinical trials as a molecule to replace opioids in postoperative pain management.

Encouraged by the discovery of oliceridine[80], we undertook a large library docking screen against the crystal structure of the μ-opioid receptor[81], seeking new biased agonists. Though we could not expect docking to reveal subtle bias-conferring interactions, we hoped to find new agonist chemotypes and be able to experimentally select for those with biased signaling. Over 3 million commercially available lead-like molecules were docked, and 23 high-ranking molecules were selected for testing[82]. Seven molecules had K_i values ranging from 2.5 to 14 μM. Though these activities were modest, the scaffolds were novel, and several were readily optimized. This rapidly led to compound PZM21 (Fig. 5), a 3.8 nM potent partial agonist of the μ-opioid receptor, activating the Gi pathway with little arrestinergic signaling.

Again, consistent with the β-arr knockout studies[79], the G-protein bias of PZM21 led to analgesia in a mouse model with little respiratory depression. Unexpectedly, this analgesia appeared to be strictly central, reducing affective pain without modulating reflex pain[82]. Just as surprisingly, PZM21, in contrast to classic opioids such as morphine, did not provoke locomotion in a closed-field mouse assay, nor did it lead to reinforcing behavior in a 10-d conditioned-place preference assay. These unusual effects at least partially reflect the insistence for using novel chemotypes at the start of the campaign.

Other biased agonists with efficacy *in vivo*

Trevena has reported on TRV250, a G-protein-biased δ-opioid receptor agonist that has efficacy against migraine and chronic pain without the serious liabilities of conventional delta-opioid receptor (DOR) agonists[83]. They have also reported TRV027, a new angiotensin II (ATII) agonist that is actually β-arr biased. TRV027 improves cardiac cell survival following acute and chronic heart failure in animals[84], although a recent clinical trial did not meet its metrics. Meanwhile, β-arr-biased D2 agonists are being evaluated for treating schizophrenia and related disorders[42,85], whereas G-protein-biased κ-opioid receptor

agonists have favorable therapeutic actions with fewer dysphoric-like side effects than is typical for unbiased κ-opioid agonists[86,87].

New opportunities for biased agonists

Biased ligands can reveal hidden biology in even well-established targets. Given that GPCRs drive multiple signaling pathways, most are candidates for such ligand discovery. Several of the centrally acting aminergic receptors stand out, including the D1 and D4 dopamine receptors, the $5HT_{2A}$ and $5HT_{2C}$ serotonin receptors, and several opioid receptor subtypes. For example, activation of G-protein signaling by the DRD1 (ref. 88) and by the DRD4 receptors has been mooted as a target for cognition enhancement in schizophrenia and ADHD, respectively. Similarly, a G-protein-biased DRD1 agonist could treat Parkinson's disease, reducing dyskinetic and hypertensive effects associated with the arrestinergic pathway for this receptor; Pfizer has reported biased D1 receptor agonists for this indication.

There has been a long-time interest in biased agonists for serotonin receptors. Intriguingly, receptor internalization and down-regulation by the atypical antipsychotic drug clozapine, active on serotonin receptors, is considered to be essential for its unique actions in schizophrenia[89]. Identifying the structural features responsible for clozapine's apparent agonist activity on receptor internalization could lead to 5-HT_{2A} modulators with efficacies in psychiatric disorders[90]. This is supported by the recent approval of the $5HT_{2A}$ inverse agonist pimavanserin (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm498442.htm) for treating psychosis associated with Parkinson's disease. Meanwhile, at the paralogous 5-HT_{2C} receptor, β-arr interactions appear to be strongly modulated by agonists, RNA editing, and constitutive activity[91]. Functional selectivity here may be responsible for some unique actions of antipsychotic drugs *in vitro*[92] and of hallucinogens *in vivo*[93]. As unbiased 5-HT_{2C} agonists may have their therapeutic actions blunted with repeated dosing via engagement of the arrestin pathway (inducing desensitization, internalization, and receptor downregulation[91]), G-protein-biased agonists may yield compounds with therapeutic advantages.

Novel ligands conferring new GPCR pharmacology

Campaigns against new GPCRs and new allosteric sites on GPCRs will continue to drive the discovery of novel ligands. Acting at previously unexplored targets, or as rheostats for endogenous signaling, such ligands are likely to confer new biology. Less expected is the return to well-precedented receptor sites with ligands that bias toward one of several signaling pathways that have previously been entangled. Though new chemotypes for these sites are not guaranteed to confer new biology, they often do, perhaps exploiting new conformations from a manifold of active and inactive GPCR states. The recent confluence of new signaling assays[40], new crystal structures, and vast new compound libraries that may be computationally interrogated for receptor fit, augurs a renaissance in GPCR pharmacology and chemical biology.

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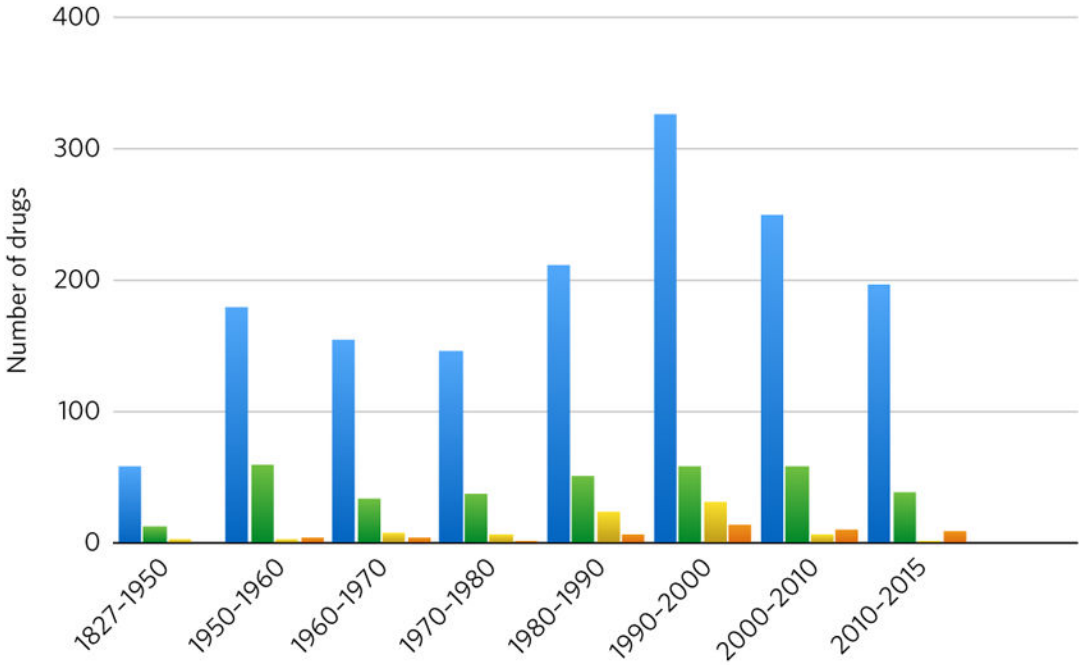

Figure 1. GPCR drugs as a percentage of all drugs, by decade of introduction

Total number of drugs introduced (blue); number of drugs targeting GPCRs as primary mechanism-of-action targets (green); number of non-GPCR drugs that also act on a GPCR at levels higher than 1 μM (yellow), number of non-GPCR drugs predicted to act on GPCRs, with similarity ensemble approach (SEA)-based[104] E-values better than 10^{-50} (orange).

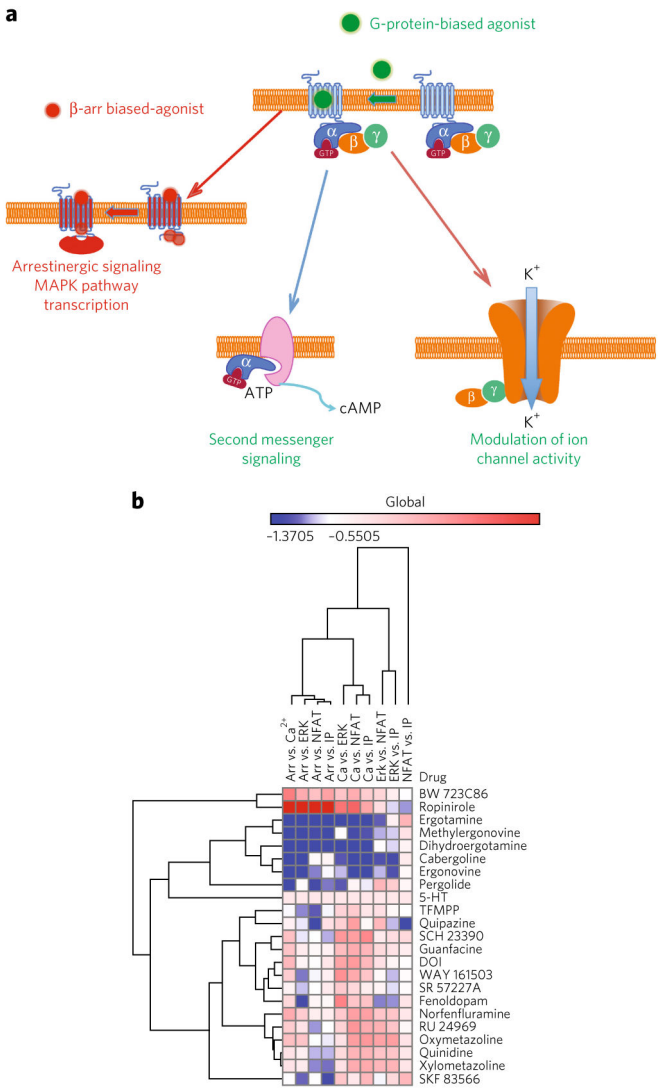

Figure 2. GPCRs may activate multiple downstream signaling pathways: role of biased signaling

(**a**) Shown are typical pathways modulated by G-protein and arrestin (β-arr) biased ligands, which lead to different intracellular signaling pathways and distinct *in vivo* activities. MAPK, mitogen-activated protein kinase; cAMP, cyclic AMP. (**b**) A heat map for ligand functional selectivity against the $5HT_{2B}$ receptor reveals distinct ligand-specific patterns. Shown are calculated estimates of bias for $5HT_{2B}$ agonists at downstream targets. Data are from ref. 105, and estimates of bias were calculated using the operational model and displayed on a heat map. ERK, extracellular signal-regulated kinase; IP, inositol phosphate; NFAT, nuclear factor of activated T cells.

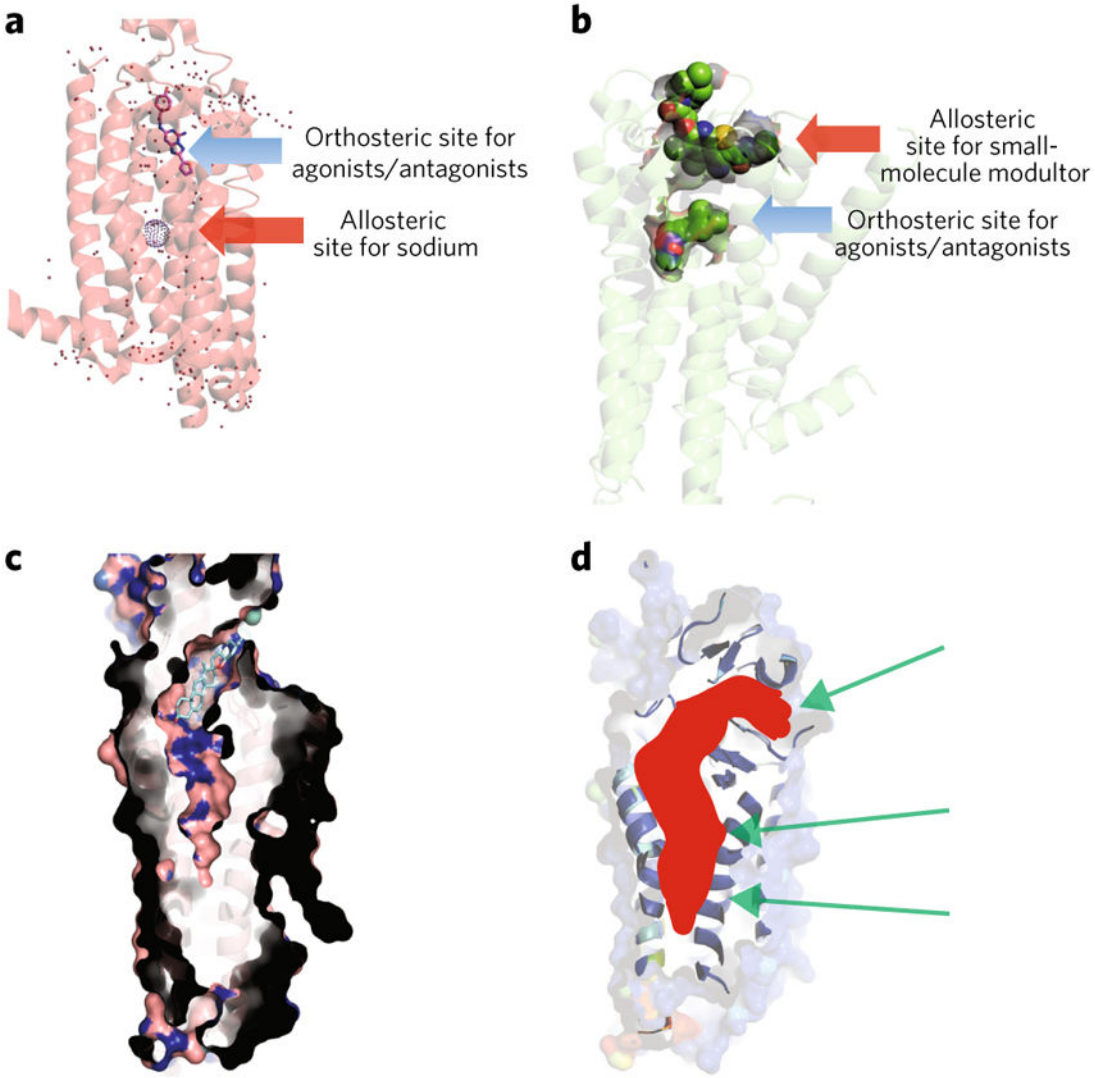

Figure 3. Multiple allosteric sites for GPCRs

(**a**) Site for the negative allosteric modulator (NAM) sodium in prototypical GPCRs, revealing its conserved location. The small orange and purple dots represent water molecules. (**b**) The locations of a muscarinic receptor positive allosteric modulator (PAM) and an orthosteric ligand. (**c**) Smoothened, and the location of various allosteric ligands for which crystal structures have been solved. (**d**) The elongated pocket defined by these ligands; the arrows illustrate sites for candidate Smoothened ligands.

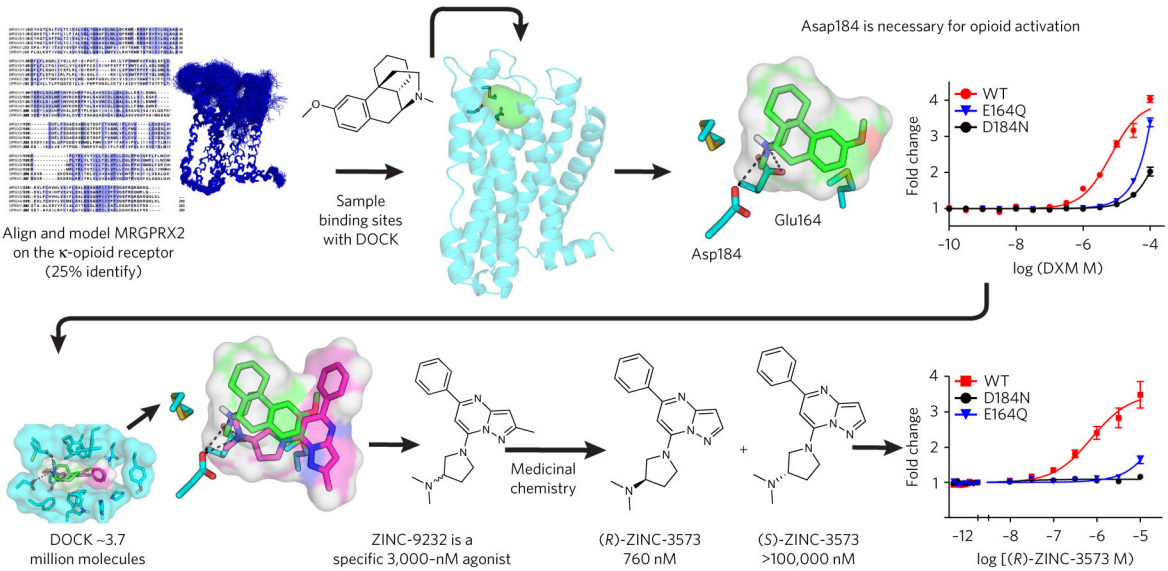

Figure 4. Physical and docking screens to deorphanize mRGPRX2

A PRESTO-Tango screen of 8,000 drugs and reagents showed opioids as agonists of MRGPRX2. Receptor structure modeling followed by a large-library docking screen revealed a 0.7 μM specific agonist of MRGPRX2 whose stereoisomer is inactive. The molecule, ZINC-3573, is active in a cell-based degranulation assay, whereas it's stereoisomer is inactive both in signaling assays and in cell culture. The two molecules are openly available as a probe pair from Sigma (http://www.sigmaaldrich.com/catalog/search?interface=All&term=(R)-ZINC-3573&N=0&focus=product&lang=en&region=US). Reprinted from ref. 44.

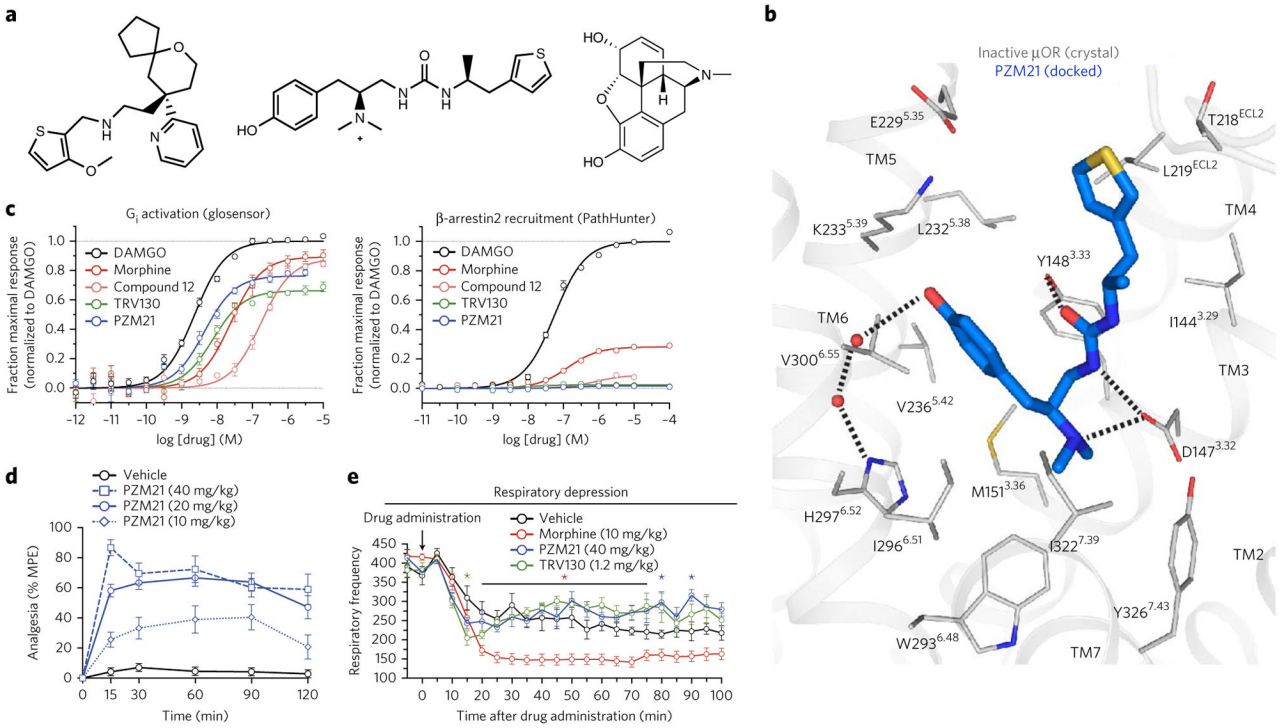

Figure 5. Novel biased agonists for the μ-opioid receptor

(**a**) The investigational new drug oliceridine (TRV130, left) and the lead compound PZM21 (center) do not resemble classical agonists like morphine (right), but both confer analgesia without causing some of the dose-limiting side effects of the classic opioid drugs. (**b**) The docked pose of PZM21 in the μ-opioid receptor (μOR). Dashed lines represent hydrogen bond interactions and red spheres represent water molecules. (**c**) G protein (left) biased signaling vs. β-arrestin biased signaling (right) of PZM21. DAMGO is a peptide agonist of the μ opioid receptor, and compound 12 is a precursor to PZM21. Error bars from replicate experiments, as described in ref. 82. (**d**) Mouse analgesia of PZM21 vs. vehicle. MPE, maximum possible effect. (**e**) Respiratory depression conferred by PZM21, morphine, TRV130, and vehicle. Curves for PZM21 are shown in blue, for morphine in red, for TRV130 in green, and for vehicle in black. **b–e** reprinted from ref. 82.

Table 1

Selected large-library docking screens against GPCRs

Target	Hit rate (active/tested)	Best hit (μM)
H1 histamine[94]	73% (19/26)	0.006
β2-AR[75,95]	24% (6/25)	0.009
A2a adenosine[96]	41% (23/56)	0.032
A2a active state[97]	45% (9/20) (only antagonists found)	0.016
Dopamine D3 (ref. 98)	56% (14/25)	0.006
Dopamine D3 (allosteric)[98]	32% (8/25)	0.5
Dopamine D3 (ref. 41)	20% (5/25)	0.3
Dopamine D3[a] (ref. 41)	23% (6/26)	0.2
Dopamine D2 (ref. 77)	46% (10/21)	0.058
Muscarinic M2/M3 (ref. 76)	56% (11/19)	1.2
GLR[94]	8.5% (2/23)	1.9
mGlu1[a] (ref. 99)	14% (5/35)	10.2
5HT6[a] (ref. 100)	17% (6/36)	0.1
Histamine H4[a] (ref. 101)	18% (15/85)	8.4
A1 adenosine[a][102]	21% (8/39)	0.4
κ-opioid[103]	18% (4/22)	7.2
μ-opioid[82]	30% (7/23)	2.5
GPR68 (ref. 45)	33% (5/15)	10
MRGPRX2 (ref. 44)	6% (1/15)	3

[a]Homology model; PAM affinity not fully characterized.